

africanus, Bacillus caldotenax, and Bacillus stearothermophilus.

5. (Amended) The recombinant thermostable DNA polymerase of claim 2 wherein said polymerase is from a Thermus species.

6. (Amended) The recombinant thermostable DNA polymerase of claim 5 which is characterized in that

AI a) in its native form said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaIleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or Gly, "Xaa" at position 4 is any amino acid, and "Xaa" at position 6 is Ser or Ala; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

7. (Amended) The recombinant thermostable DNA polymerase of claim 5 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

8. (Amended) The recombinant thermostable DNA polymerase of claim 7 wherein said "Xaa" at position 4 is mutated to Lys.

9. (Amended) The recombinant thermostable DNA polymerase of claim 2 wherein said polymerase is from a Thermotoga species.

10. (Amended) The recombinant thermostable DNA polymerase of claim 9 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid and "Xaa" at position 7 is Val or Ile; and

A1 b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

16. (Amended) The nucleic acid sequence of claim 15 which is characterized in that

A2 a) in its native form said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaalleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or Gly, "Xaa" at position 4 is any amino acid, and "Xaa" at position 6 is Ser or Ala; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

17. (Amended) The nucleic acid sequence of claim 15 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaalleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

18. (Amended) The nucleic acid sequence of claim 17 wherein said "Xaa" at position 4 is mutated to Lys.

20. (Amended) The nucleic acid sequence of claim 19 which is characterized in that

A3 a) in its native form said polymerase comprises the amino acid sequence LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid and "Xaa" at position 7 is Val or Ile; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

21. (Amended) A method of DNA sequencing which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

A3 LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile, and

ii) said polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;

b) providing a dye-terminator labeled with a negatively charged fluorescent dye; and

c) performing a dye-terminator sequencing reaction.

A4 26. (Amended) The method of claim 25 wherein said amino acid sequence comprises LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

A5 29. (Amended) The method of claim 28 wherein said amino acid sequence comprises LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

A6 31. (Amended) A method of producing labeled DNA which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile, and

ii) said polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;

b) providing a nucleotide labeled with a fluorescein family dye; and

c) performing a DNA synthesis reaction.

32. (Amended) A method of producing labeled primer extension products which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile,

ii) said polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase,

iii) said polymerase also comprises the second amino acid sequence

SQIXLR(V/I) (SEQ ID NO: 18) where "X" is any amino acid except E,

iv) said polymerase has a level of discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;

b) providing a ribonucleotide labeled with a fluorescein family dye; and

c) performing a primer extension reaction.

33. (Amended) A kit for DNA sequencing which comprises:

a) a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile, and

ii) said polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase; and

b) a terminator labeled with negatively-charged fluorescent dye.

36. (Amended) The kit of claim 35 wherein said "Xaa" at position 4 is Lys.

37. (Amended) The kit of claim 34 wherein said amino acid sequence comprises LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

38. (Amended) The kit of claim 37 wherein said "Xaa" at position 4 is Arg.

A7  
39. (Amended) A kit for DNA sequencing which comprises:

a) a mutant thermostable DNA polymerase characterized in that

i) in its native form said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 4, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 7 of this sequence is Val or Ile;

ii) said amino acid sequence is mutated, except that "Xaa" at position 4 is not mutated to Glu; and

iii) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.

A8  
42. (Amended) The kit of claim 41 wherein said "Xaa" at position 4 is mutated to Lys.

44. (Amended) The kit of claim 43 wherein said "Xaa" at position 4 is Arg.

A9  
45. (Amended) A kit for producing labeled DNA which comprises:

a) a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 7), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile,

ii) said polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase; and



b) a nucleotide labeled with a negatively-charged fluorescent dye.

46. (Amended) The kit of claim 45 wherein said amino acid sequence comprises LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:14), whereby "Xaa" at position 4 is any amino acid except Glu.

47. (Amended) The kit of claim 45 wherein said "Xaa" at position 4 is Lys.

48. (Amended) The kit of claim 45 wherein said amino acid sequence comprises LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 15), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

49. (Amended) The kit of claim 48 wherein said "Xaa" at position 4 is Arg.

50. (Amended) A kit for producing labeled primer extension products which comprises:

a) a thermostable DNA polymerase which is characterized in that

i) in its native form, the polymerase comprises the first amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile;

ii) the polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;

iii) the polymerase also comprises the second amino acid sequence SQIXLR(V/I) (SEQ ID No: 18) where "X" is any amino acid except E;

iv) the polymerase has a level of discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase; and

b) a ribonucleotide labeled with a fluorescein family dye.

51. (Amended) The kit of claim 50 wherein said amino acid sequence comprises

LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

52. (Amended) The kit of claim 51 wherein said "Xaa" at position 4 is Lys.

53. (Amended) The kit of claim 50 wherein said amino acid sequence comprises LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

54. (Amended) The kit of claim 53 wherein said "Xaa" at position 4 is Arg.